

**Amendments to the Specification:**

Please replace the paragraph beginning at page 13, line 16, with the following rewritten paragraph:

A1  
The selection system described above is most appropriate for use in selecting mutants with the desired substrate specificity from a heterogeneous population of ~~heterogenous~~ mutant fatty acid desaturase molecules. By transforming a population of mutated nucleic acid sequences into the auxotroph host cells, entire libraries of mutants can be screened for the ability to complement the MH13 auxotrophy.

Please replace the paragraph beginning at page 16, line 18, with the following rewritten paragraph:

A2  
All five mutant proteins listed in ~~Table 3~~ Table 4 have the amino acid substitutions T117R and G188L, in combination with various substitutions at the remaining four positions. The fact that these two mutations are the optimal changes at their respective positions for reducing chain length specificity suggests that they are likely the primary determinants of the altered specificity in com2. The observation that several other mutants containing this pair of mutations have lower specific activity suggests that the combination of mutations at the remaining four randomized sites can also affect the specific activity of the mutants. This conservation suggests that the substitutions T117R and

*Cal*  
*A2*  
G118L are responsible for the change in substrate specificity of the five mutants in ~~Table 3~~ Table 4. A mutant desaturase with the combination of T117R and G118L substitutions is expected to have enhanced activity for one or more substrates with 16 or fewer carbons.

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Please replace the paragraph beginning at page 17, line 11, with the following rewritten paragraph:

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*A3*  
Another aspect of the present invention is a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has a subset of the amino acid substitutions of a mutant protein listed in ~~Table 3~~ Table 4. Such a mutant is expected to also have altered activity towards the different substrates. The present invention encompasses mutant castor  $\Delta^9$ -18:0-ACP desaturase proteins which have between 1 and 6 of the amino acid substitutions of the com2 mutant, in any possible combination. In a preferred embodiment, the combination includes at least three of the amino acid substitutions of the com2 mutant. Preferably two of these amino acid substitutions are T117R and G188L. In addition, the present invention is intended to encompass mutant  $\Delta^9$ -18:0-ACP desaturase proteins which have between 1 and 6 of the amino acid substitutions of the com3 mutant, the com4 mutant, the com9 mutant, or the com10 mutant,

CAH  
A3  
respectively, in any possible combination. In a preferred embodiment, the combination includes at least three of the amino acid substitutions, of the mutant. Preferably two of these substitutions are T117R and G188L. Also included in the present invention are mutants which have the above listed amino acid substitutions, and combinations thereof, in combination with any other amino acid substitutions, insertions or deletions. These additional substitutions, insertions or deletion, may be silent (e.g. do not affect function of the enzyme) or may further alter enzyme function.

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Please replace the paragraph beginning at page 29, line 13, with the following rewritten paragraph:

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A4  
The nucleic acid sequence for castor  $\Delta^9$ -18:0-ACP desaturase was subjected to one of two types of mutagenesis, site directed or random mutagenesis, prior to introduction into the MH13 cells. PCR was used in site directed mutagenesis to randomize a targeted codon corresponding to a specified residue in the amino acid sequence of the castor  $\Delta^9$ -18:0-ACP desaturase. Target codons corresponding to Met 114, Leu 118, Pro 179, and Gly 188 were each subjected to independent randomization. Previous studies (Cahoon, et al. (1997)) had indicated that these residues are located adjacent

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to the substrate binding cavity and that replacing some of those amino acids in the *T. alata*  $\Delta^6$ -16:0 desaturase or in the castor  $\Delta^9$ -18:0 desaturase with bulkier or less bulky amino acids could affect substrate specificity *in vitro*. The methods of the present invention allowed for an unbiased substitution of all 20 amino acids into these positions but required that the mutation ~~have an affect on~~ the *in vivo* substrate specificity of the desaturase. The mutagenesis reactions yielded four populations, each one comprising a library of coding sequences with substitution mutations consisting of all 20 potential amino acids at the designated mutation site.

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